REMARKS

Claim Amendments

Claims 114, 122, 130, 138, 146, 148, 150, 152, 160, 168, 176, 184, 186, 188, 190, 199, 208, 217, 226, 229 and 232 have been amended to specify that the reference RNA sequence and the (selected) target viral RNA sequence are of similar length. These claims have also been amended to specify that the reference RNA sequence and the (selected) target viral RNA sequence are capable of being amplified by the same oligonucleotides. Support for the former amendment can be found in the disclosure in the present application in which the maxigene and minigene constructs differ from the target viral RNA sequence by about 20 bases (see page 6, lines 19-21). Support for the latter amendment can be found at page 6, lines 15-18. Applicants submit that these claims do not constitute new matter and their entry is requested.

Summary of the Claims

The claims comprise three sets of claims, each directed to one of the reference RNA sequences. These sets of claims are summarized as follows.

Claims 114-151 are directed to processes for quantitation of a target viral RNA sequence in a sample, an amplification reaction mixture (claims 146-147), a reverse transcription reaction mixture (claims 148-149) and a kit (claims 150-151). The process involve the simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 114-121 and 130-137) or involve first a simultaneous reverse transcription and then a simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 122-129 and 138-145). The reference RNA sequence of claims 114-151 is a reference RNA sequence that consists of the target viral RNA sequence with a multibase insert into a site within the target viral RNA sequence

Claims 152-189 are directed to processes for quantitation of a target viral RNA sequence in a sample, an amplification reaction mixture (claims 184-185), a reverse transcription reaction mixture (claims 186-187) and a kit (claims 188-189). The process involve the simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 152-159 and 168-175) or involve first a simultaneous reverse transcription and then a simultaneous

amplification of a target viral RNA sequence and reference RNA sequence (claims 160-167 and 176-183). The reference RNA sequence of claims 152-189 is a reference RNA sequence that consists of the target viral RNA sequence with a multibase deletion from a site within the target viral RNA sequence.

Claims 190-234 are directed to processes for quantitation of a target viral RNA sequence in a sample, an amplification reaction mixture (claims 226-228), a reverse transcription reaction mixture (claims 229-231) and a kit (claims 232-234). The process involve the simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 190-198 and 208-216) or involve first a simultaneous reverse transcription and then a simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 199-207 and 217-225). The reference RNA sequence of claims 190-234 is a reference RNA sequence that comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence.

Support for Claim Language

As noted above, support for the language that the reference RNA sequence and the (selected) target viral RNA sequence are similar in length can be found in the disclosure of the maxigene and minigene in the present application, in which the reference RNA sequence differs from the target viral RNA sequence by about 20 bases (page 6, lines 19-21) and by the use of a small insertion in the target viral RNA sequence (page 7, lines 12-15.

Summary of the Invention

The present invention is directed to a method for the quantitation of target viral RNA in a sample by simultaneously amplifying a target viral RNA sequence and a known quantity of a reference RNA sequence as an internal standard. That is, the target viral RNA sequence, if present, and the reference sequence are simultaneously amplified in the same reaction mixture. The quantity of target viral RNA present in the sample is determined by comparing the amount of the amplified target viral RNA and the amount of the amplified reference RNA based on the known quantity of reference RNA added as an internal control. The reference RNA sequence

may be (a) a reference RNA sequence that consists of the target viral RNA sequence with a multibase insert into a site within the target viral RNA sequence, (b) a reference RNA sequence that consists of the target viral RNA sequence with a multibase deletion from a site within the target viral RNA sequence or (c) a reference RNA sequence that comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence. In each instance, the reference RNA sequence and the target viral RNA sequence are of similar length and are capable of being amplified by the same oligonucleotides.

Priority

According to the first paragraph of the specification, the present application is a continuation-in-part of three applications, Serial No. 07/355,296, filed 22 May 1989, Serial No. 07/143,045, filed 12 January 1988 and Serial No. 07/148,959, filed 27 January 1988. Thus, the present application claims priority to each of these three applications. As discussed below, Applicants submit that they are entitled to a priority date of at least 27 January 1988 for the amended claims.

With respect to claims 114-151, Applicants note that the Examiner has indicated that there is support in the parent applications for a multibase insert in which the reference RNA sequence and the target viral RNA sequence have similar lengths. For example, the '045 application specifies a small insertion (page 8, lines 12-15) and the '959 application specifies a maxigene of about 22 nucleotides (page 3, lines 27-31). The Examiner has also indicated that there is support in the parent application for the reference RNA sequence and the target viral RNA sequence being capable of being amplified by the same oligonucleotides. For example, see page 3, lines 23-26 of the '959 application. This identification to support in the parent applications is in addition to the support that was set forth in the Amendment filed 15 December 2005. Therefore, Applicants submit that this subject matter has priority and benefit to the '959 application. Thus, Applicants submit that the priority date for this subject matter is the filing date of the '959 application, i.e., 27 January 1988.

With respect to claims 152-189, Applicants note that reference RNA sequence of these claims, i.e., a minigene, does not appear *in haec verba* in the parent '959 application. However,

Applicants submit that this reference RNA sequence is supported by the parent application in its disclosure that the target viral sequence and the reference RNA sequence are distinguishable by size. See page 4, lines 13-14 of the '959 application. The size that is shown in the '959 application for the reference RNA sequence is similar in length to the target viral RNA sequence. The Examiner has also indicated that there is support in the parent application for the reference RNA sequence and the target viral RNA sequence being capable of being amplified by the same oligonucleotides. For example, see page 3, lines 23-26 of the '959 application. This identification to support in the parent applications is in addition to the support that was set forth in the Amendment filed 15 December 2005. Therefore, Applicants submit that this subject matter has priority and benefit to the '959 application. Thus, Applicants submit that the priority date for this subject matter is the filing date of the '959 application, i.e., 27 January 1988.

With respect to claims 190-234, Applicants note that maxigene contains an insert in the target viral RNA sequences (e.g., see page 3, lines 27-28 of the '959 application). Because the insert sequence is not a target viral RNA sequence, the "maxigene" comprises target viral RNA sequence and non-target viral RNA sequence. The amplified target viral RNA sequence and the amplified reference sequence are distinguishable by size. See page 4, lines 13-14 of the '959 application. Reference sequences with a multibase insert or a multibase deletion are capable of being distinguished from the target viral RNA sequence by size. Thus, the '959 application a reference RNA sequence that contains target viral RNA sequence and non-target viral RNA sequence. A similar reference sequence is shown in the 'the '045 application, i.e., a small insertion (page 8, lines 12-15 of the '045 application). The Examiner has also indicated that there is support in the parent application for the reference RNA sequence and the target viral RNA sequence being capable of being amplified by the same oligonucleotides. For example, see page 3, lines 23-26 of the '959 application. This identification to support in the parent applications is in addition to the support that was set forth in the Amendment filed 15 December 2005. Therefore, Applicants submit that this subject matter has priority and benefit to the '959 application. Thus, Applicants submit that the priority date for this subject matter is the filing date of the '959 application, i.e., 27 January 1988.

New Matter Rejection

The Examiner rejected claims 114-234 under 35 U.S.C. § 112, first paragraph for new matter. Applicants submit that the amendment of the claims to specify that the reference RNA sequence and the target viral RNA sequence are similar in length and are capable of being amplified by the same oligonculeotides obviates this rejection obviates this rejection. Applicants submit that the amended claims no longer encompass any size reference RNA sequence. Applicants note that the application clearly discloses quantitation of a target viral RNA sequence using a reference sequence which has a similar length as the target viral RNA sequence and which is capable of being amplified by the same oligonucleotides.

In view of the amendments to the claims and the above remarks, it is submitted that claimed subject matter is fully described in the specification. Withdrawal of this rejection is requested.

Enablement Rejection

The Examiner rejected claims 114-234 under 35 U.S.C. § 112, first paragraph for lack of enablement for the scope of the claimed subject matter. Applicants submit that the amendment of the claims to specify that the reference RNA sequence and the target viral RNA sequence are similar in length and are capable of being amplified by the same oligonucleotides obviates this rejection. Applicants submit that the amended claims no longer encompass any size reference RNA sequence. Applicants note that the application clearly shows quantitation of a target viral RNA sequence using a reference sequence which has a similar length as the target viral RNA sequence and which is capable of being amplified by the same oligonucleotides.

In view of the amendments to the claims and the above remarks, it is submitted that the claimed subject matter is fully enabled by the specification. Withdrawal of this rejection is requested.

Anticipation Rejection

The Examiner rejected claims 146-151 and 229-234 under 35 U.S.C. § 102(a) as being anticipated by Murakawa et al. (*DNA* **7**:287-295, 1988). Applicant submits that the amended

claims are entitled to the priority date of the '959 application, i.e., 27 January 1988. This date is prior to the date of Murakawa et al., and thus Murakawa et al. is not prior art against the amended claims.

In view of the amendments to the claims and the above remarks, it is submitted that the claimed subject matter is entitled to a priority date of 27 January 1988 and is not anticipated by Murakawa et al. Withdrawal of this rejection is requested.

Obviousness Rejections

The Examiner rejected claims 115-145 and 190-228 under 35 U.S.C. § 103(a) for being obvious over Murakawa et al. in view of Chelly et al. (*Nature* **333**:858-860, 1988). Applicant submits that the amended claims are entitled to the priority date of the '959 application, i.e., 27 January 1988. This date is prior to the date of Murakawa et al. and Chelly et al. Thus, neither Murakawa et al. nor Chelly et al. is prior art against the amended claims.

In view of the priority date to which the amended claims is entitled, it is submitted that the claimed subject matter is not obvious over the cited references. Withdrawal of this rejection is requested.

Furthermore, it is submitted that the amended claims are not obvious from the cited references. Specifically, the Examiner contends that Chelly et al. discloses adding a reference RNA to a sample and that quantitation of the DNA of interest is determined. The Examiner is incorrect in her reading of this reference. Chelly et al. describes the simultaneous amplification of a heterologous reference sequence, i.e., aldolase A, and a target sequence, i.e., dystrophin. Instead, the sample that is amplified by Chelly et al. contains both dystrophin and aldolase A. Chelly et al. clearly does not add the aldolase A (reference sequence) to the sample. Because Chelly et al. does not add a reference RNA, it does not add a known quantity of the reference RNA. Thus, the Examiner's interpretation of an added reference RNA in Chelly et al. is incorrect. Applicants also note that this interpretation is counter to facts found by the Board in the Wang v. Murakawa interference (Patent Interference No. 105,055; see Memorandum Opinion and Order (Decision on Wang preliminary motion 1) dated 5 November 2003).

In addition, Applicants submit that Chelly et al. does not describe a process for quantitation of a target sequence. Chelly et al. specifically states that the absolute value of dystrophin mRNA cannot not be determined directly but that a relative figure can be obtained by comparing with the aldolase A internal standard. *See*, Figure 3 of Chelly et al. The internal standard is a standard that is present in the samples used for amplification and is not a standard that is added to the sample in a predetermined amount for simultaneous amplification and quantitation. This lack of being able to determine an absolute amount of the target sequence is in direct contrast to the present invention which provides for the quantitation of the target sequence.

More specifically, Chelly et al. also does not describe a method for the precise quantitation of a target RNA sequence, but merely describes a method for the crude estimation of the relative amount of the target dystrophin mRNA. Chelly et al. explains that aldolase A mRNA was used to check efficiency of the amplification reaction. See page 858, left column ("To check the efficiency of the procedure, we simultaneously co-reverse transcribed and co-amplified another transcript as an internal standard in the same test tube."). The calculations using the "labeled primers" to determine the relative amount of the target dystrophin mRNA in the sample (relative to total mRNA) were based on the ratio of target to standard and the estimated amount of standard mRNA in certain specific tissues. See page 859, left column ("From this value we deduced the amount of dystrophin mRNA relative to total mRNA (Table 1)."). In this deduction, Chelly et al. provides, at best, an estimate that the amount of aldolase A mRNA ranges from about "0.1-0.5% of total mRNA in skeletal muscle, and at least ten times less in other tissues." See page 859, left column. The title of Table 1 ("Estimation ...), as well as the abstract ("quantitative estimate"), confirms that Chelly et al. only estimated the amount of dystrophin mRNA in the sample. Applicants submit that a quantitative estimate is not quantitation and no quantitative amount is provided by Chelly et al. Chelly et al. also states that the absolute value of dystrophin mRNA can not be determined directly but a relative figure can be deduced. See legend to Figure 3 under "Methods." Chelly et al. requires the use of ratios to measure expression of the target dystrophin mRNA because the standard, aldolase A, is not used in a known initial amount, as required by the present invention. It is the use of a known quantity of the reference RNA sequence in the claimed method, an element missing from Chelly et al.,

which enables the quantitation of target viral RNA, because the initial amount of the reference sequence is known.

The labeled primers were used to determine the efficiency of the reaction and to calculate the ratio of dystrophin mRNA to aldolase A mRNA in the sample. See, page 859, top of left column. The labeled primers were not used to quantitate the amount of target RNA in the sample. This calculation only provides a ratio of dystrophin mRNA to aldolase RNA and does not provide any quantitation of the dystrophin mRNA. The ratio is useful in identifying tissues that express dystrophin.

From the above analysis of Chelly et al., it is apparent that Chelly et al.'s experimental objective was to provide a relative estimate of the amount mRNA present in a sample, such that the estimate was, at best, a comparative estimate. There is no attempt to use PCR to quantitate actual amounts of target. This objective differs from Applicants' objective which was to use PCR to quantitate a target viral RNA. Because Chelly et al.'s objective differs from Applicants' objective, it is evident that Chelly et al. could not motivate or suggest the claimed invention even if the critical elements of the present invention were disclosed therein.

Chelly et al. only discloses the determination of a relative quantity of a target RNA sequence. It does not disclose the absolute quantitation of the target sequence. Because Chelly et al. does not describe such quantitation, it does not supply the element missing from Murakawa et al. that has been noted by the Examiner. Thus, Applicants submit that the combination of Murakawa et al. and Chelly et al. does not render the invention obvious.

Finally, Applicants submit that there is no motivation or suggestion to combine the prior art in the manner proposed by the Examiner. According to the Examiner, Murakawa et al. discloses a reference sample that is a target sequence with an insert that is similar in length as the target sequence. Chelly et al. discloses a reference sequence which is totally heterologous to the target sequence, i.e., the reference sequence that Chelly et al. uses contains no sequence found in the target sequence. Thus, Applicants submit that there would be no motivation to combine Chelly et al. with Murakawa et al.

In view of the amendments to the claims and the above remarks, it is submitted that the claims are not obvious over the combination of Murakawa et al. in view of Chelly et al. Withdrawal of this rejection is requested.

The Examiner also rejected claims 152-189 under 35 U.S.C. § 103(a) for being obvious over Murakawa et al. in view of Chelly et al. further in view of Arya (*Proc Natl Acad Sci USA* **84**:5429-5433, 1987). Applicants submit that Arya does not add anything to the deficiencies of the combination of Murakawa et al. and Chelly et al. that were described above. Thus, Applicants submit that the claimed invention is not obvious from the cited prior art.

In view of the amendments to the claims and the above remarks, it is submitted that the claimed subject matter is not obvious over the combination of Murakawa et al. in view of Chelly et al. further in view of Arya. Withdrawal of this rejection is requested.

Concluding Comments

In view of the above amendments and remarks, it is submitted that the claims are fully supported by the instant application and are patentable over the prior art of record. Reconsideration of this application and early notice of allowance is requested. The Examiner is invited to telephone the undersigned if it will assist in expediting the prosecution and allowance of the instant application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By _____

Jeffrey L. Ihnen
Registration No. 28,957
1425 K Street, N.W., Suite 800
Washington, D.C. 20005
Telephone No.: (202) 783-6040
Facsimile No.: (202) 783-6031